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Blast Related Neurotrauma: A review of Cellular Injury

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Abstract: Historically, blast overpressure is known to affect primarily gas-containing organs such as the lung and ear. More recent interests focus on its ability to cause damage to solid organs such as the brain, resulting in neurological disorders. Returning veterans exposed to blast but without external injuries are being diagnosed with mild traumatic brain injury (Warden 2006) and with cortical dysfunction (Cernak *et al* 1999). Decades of studies have been conducted to elucidate the effects of primary blast wave on the central nervous system. These studies were mostly concerned with systemic effects (Saljo *et al* 2000-2003; Kaur *et al* 1995-1997, 1999; Cernak *et al* 1996, 2001). The molecular mechanism of blast-induced neurotrauma is still poorly understood. This paper reviews studies related to primary blast injury to the nervous system, particularly at the cellular level. It starts with a general discussion of primary blast injury and blast wave physics, followed by a review of the literature related to 1) the blast wave/body interaction, 2) injuries to the peripheral nervous system, 3) injuries to the central nervous system, and 4) injury criteria. Finally, some of our preliminary data on cellular injury from *in vitro* and *in vivo* studies are presented. Specifically, we report on the effects of overpressure on astrocytes. In the discussion, possible mechanisms of blast-related brain injury are discussed, as well as the concerns and limitations of the published studies. A clearer understanding of the injury mechanisms at both the molecular and macroscopic (organ) level will lead to the development of new treatment, diagnosis and preventive measures.

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1 Overview

Increasing use of improvised explosive devices (IEDs) and other forms of explosives by terrorists have led to mass casualties. Bombing survivors usually have higher injury severity scores and a greater need for surgical intervention, intensive care, and longer hospitalization. They are also in the younger age group (1). Table 1 summarizes the injury statistics from some bombing events. Blast injuries often result in polytrauma and are thus a high priority concern that is also of high cost.

Blast waves from detonations affect many physiological systems 'silently,' typically the gas-containing organs. Tympanic membrane ruptures due to pressure waves (2) and eye injuries caused by high-velocity projectiles (3) are commonly seen in bombing victims. The lung is another susceptible organ to blast waves, but recent updates in protective gear have decreased the amount of lung injury currently seen. High overpressures can displace the lung more at the intercostal spaces than under the ribs, causing 'rib markings' on the blast lung (4). Fung *et al* proposed and tested the hypothesis that transient impact leads to compression waves, followed by tensile waves in the lung which overstretch the alveolar membrane and cause lung injury (5). Hemorrhage and perforation occur in gas-containing structures in the abdomen after exposure to strong blast waves (3, 6). The abrupt pressure change due to a blast can produce brain concussion or contusion. It can also cause cavitation in blood vessels, resulting in air emboli that can travel to the brain to cause a cerebral infarct (7). Neurological disorders such as insomnia, impaired

Table 1: The injury pattern and involvement of different organs (as a percentage of the total casualties) in bombing victims.

Events	**North Ireland** 1969-1977 (89)	**Civilian bus in Jerusalem** (90)	**Okalahoma** 1995 (91)	**Madrid** 2004 (92)	**Patients in VAMC, Florida** 2004-2005 (93)
Hearing loss	45	75.8	46.9	41	42
Eye injuries	-	17.5	13	16	26
Brain/ Head injury	66	14	15	12	66
Blast lungs	47	38	-	7	22
Abdominal organs	34	14	-	5	-
Fractures/ amputation	-	24.5	4.5	18	-
Burn injuries	-	17.5	15.4	18	-
Stress syndromes	-	-	-	9	52

* VAMC stands for Veterans Affairs Medical Center

concentration, memory loss and hypervigilance (8), were reported more often in those injured by blast than in those who sustain a traumatic brain injury from other mechanisms, based on the data from patients at Walter Reed Army Medical Center (WRAMC) (9). Nonetheless, these acute stress disorders were usually treated as psychiatric disorders, due to the absence of visible injury and the mild to moderate brain injury was undiagnosed in acute clinical settings. This hidden injury was usually uncovered when the sufferers encountered difficulties in daily life. Case studies showed that some of the common clinical signs in blast-injured patients include subdural hematoma, headache, blurring of vision, transient deafness and psychoneuroses (10-12). In one case reported by Sylvia *et al*, a marine suffered immediate headache, tachycardia, severe bilateral hearing loss and blurred vision after exposure to a significant blast wave, without pulmonary and abdominal injuries or tympanic membrane rupture. During the first month after blast insult, he developed vestibular impairments, a decline in intellectual functioning, and impairments in cognitive function, memory and learning ability. However, he recovered completely in four months (13). Cernak *et al* examined the effects of brain injury in blast induced casualties without external injuries. Electroencephalographic (EEG) recordings were altered in 36% of these patients, a sign of cortical dysfunction, within the first three days after injury. In addition to the common clinical symptoms, the patients experienced retrograde amnesia, psychomotor agitation and dizziness (14). Similar EEG findings were reported in returning veterans from World War II, Korean conflict and Vietnam war who were exposed to blast (15). According to WRAMC, two-thirds of the 105 injured soldiers returning from Iraq between June and October 2003 sustained a traumatic brain injury, mostly related to blast exposure. This figure was higher than that reported in other past US conflicts, despite advancements in body armor and helmet design. It has been suggested that blast waves propagated through the protective gear into the organs to cause injury (16). Past literature has largely focused on lung and gastrointestinal tract blast-related injuries, while brain injury due to exposure to primary blast is not well understood.

2 Theory of Blast Waves and Blast Wave Model

A blast wave is a particular form of shock wave generated by an explosion. For a chemical explosion in air which is of interest here, the expanding gaseous products compress the surrounding air and generate a shock wave which propagates away from the source. A classical free-field blast wave at a fixed location passed by the blast can be modeled by a Friedlander waveform, which is characterized by an instantaneous rise in pressure immediately followed by a decaying curve (Figure 1). Details are well described by Brode (17). Associated with the sudden rise in pressure of the shock front is a blast wind (dynamic pressure) due to the kinetic energy transmitted to the air particles (18). With time and distance the peak

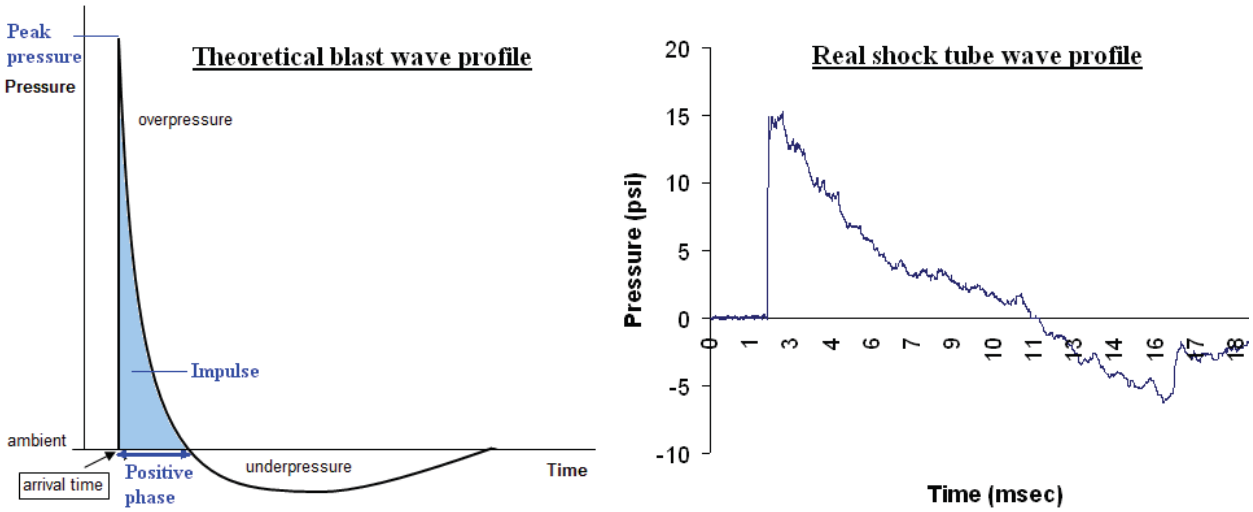

Figure 1: Friedlander wave profiles of static pressure

pressure and velocity of the blast wave weaken while it propagates. Near the source of the explosion the overpressure decreases approximately with the cube of the distance from the epicenter, but at greater distance, it decays linearly with range as an acoustic wave (18).

When a blast wave encounters a structure, loading is imparted due to shock reflection and diffraction and aerodynamic forces are generated by the blast wind. These external surface loads can cause surface deflections, damage, and global motion of the structure, and a stress-wave is propagated into the material (19). More details are provided in the next section. In the case of humans, who often have 'compliant' surfaces, the aspect of stress wave coupling as well as fluid-structure interaction (FSI) are critical to the injury problem (20). In terms of physiological significance, the critical imparted loading is the reflection/diffraction phase which is determined from the static (P_s) and dynamic pressure (P_d) of the blast wave and the geometry of the structure. Note this loading will vary dramatically around the structure and through time. Simplistically, the peak value of the imparted loading is defined by (18)

$$P_{refl} = 2P_s + 2.4P_d$$

The three parameters of primary importance are the peak value of P_{refl}, its duration, and impulse (or area under the pressure-time curve as shown shaded in Figure 1) (21). The peak overpressure is defined as the maximum value of the positive phase; the duration also refers to the positive phase, and the impulse is the area under the positive phase of the overpressure curve (see Figure 1). In the laboratory, a shock tube activated by compressed gas provides a safe and convenient way to simulate a free-field blast wave (22). Other methods to generate a blast wave or shock wave include the use of explosives and shock wave generator using micro-explosives.

3 Interaction between the blast wave and the body

A series of physical events take place instantaneously when a shock wave strikes a living body. Part of the incident shock wave is reflected against the body surface, while another fraction is deflected. Finally, the most significant fraction is absorbed and propagated through the body as a stress wave (20). A blast wave generates shear waves and stress waves when interacting with the body. Shear waves are of long-duration and low velocity, resulting from the compression of the body wall and the structures underneath. Shear waves probably account for the primary blast injury of solid abdominal viscera, mesenteries and large bowel (23). Stress waves travel at or slightly faster than the speed of sound with high amplitude. They can injure tissue in a number of ways

including spalling, implosion and pressure differentials. Spalling refers to cavitation created by reflections of a shock wave at the interface between media of different densities or acoustic impedances. It is responsible for pulmonary edema in blast lungs (24). In lungs, implosion occurs when shock wave travels through alveoli, which could be compressed to an extremely small volume and, thereby absorb a substantial amount of energy. As the shock wave passes, energy is released and the rebound expansion of each gas pocket causes damage to the alveolar walls, resulting in lung lacerations and hemothorax (24, 25). Stress waves also build up a pressure differential between internal organs and the outer surface of the body. Blood from pulmonary capillaries will be driven into the alveolar spaces, contributing to the pulmonary hemorrhage in blast lungs (25). In addition, a pressure differential generates an external force, which results in a sudden acceleration of the surface such as the tympanic membrane and chest wall (26). Stress waves transmitted through solid organs that have a small shear modulus and large bulk modulus do not cause significant compression, and thus there are no large displacements (27).

Whether brain injury is caused by a blast wave transmitted into the brain (primary mechanism for brain injury) or by blast-induced malfunctions of the pulmonary and circulatory systems (secondary mechanism for brain injury) is still controversial. Transmission of a pressure wave inside the brain during exposure to blast overpressure was examined in previous studies. Romba and Martin (28) recorded the pressure pattern inside the brain of dead monkeys, which were exposed to a shock wave generated by an explosive. They characterized the pressure wave inside the brain as of short duration with large pressure oscillations, which were damped out quickly. Furthermore, a large fraction of the pressure was transmitted into the brain through the skull while the pressure transmitted from the torso to the brain was of minor importance (Table 2). Extensive subarachnoid and cortical surface hemorrhage were observed in whales which were shot by grenades aimed at the thorax while being hunted in Norway (29). The authors suggested that the blood vessels in the meninges and brain tissue were ruptured due to the pressure propagating supersonically in all directions inside the body, mostly via fluid pathways. A study using the rabbit revealed that shock wave energy was transmitted to the brain directly through the skull without an appreciable change in amplitude and waveform (30). A recent study (31) utilized a miniature fiber optic pressure transducer to record the pressure in a cerebral ventricle of a living rat exposed to a low-level blast generated by a shock tube. In agreement with previous studies, the peak overpressure inside the brain was just slightly lower (about 2 kPa) than that in air. Interestingly, the pressure wave recorded with the rat head facing the blast was damped slowly, while in the position perpendicular to the side of the head, the wave dropped immediately after reaching the peak. Thus, impact direction affects the pressure inside the head and hence the severity of injury in the rat. In these studies, the resulting pressures were found to be modified to a lesser extent than the air-filled organs. The more homogeneous nature of the brain may account for these observations (27).

4 Injury thresholds for blast-induced neurotrauma

The standards for primary blast injury criteria were developed by Bowen *et al* in 1968 (32). Their measurements were based on peak side-on pressure. It was estimated that the threshold for lethality was approximately 6900 to 8300 kPa and lethal dose 50% (LD_{50}) was 900 to 1240 kPa in man exposed to a short-duration shock wave (33). Despite the wide use of Bowen's criteria in the literature, it was found to be overly conservative due to the large systematic error in the experiments (34). Phillips *et al* addressed the importance of establishing the primary blast injury criteria in a confined space, where sheep were exposed to complex blast waves (35). A study using sheep and swine showed that the injury threshold was lower under repeated blasts (5 blasts) compared to that for a single blast, indicating that a series of shock wave reverberations resulted in injury at lower peak overpressures (36). Simi-

Table 2: Summary of studies in the literature related to pressures developed inside the brain during exposure to blast wave

Animal	Condition	Location of pressure sensors	Charge distance (m)	Av. pressure (kPa)	Av. positive duration (ms)	Ref
Monkey	Whole body was exposed in air	Air	2.16	66.88	1.6	(28)
		Brain	2.16	70.33	2	
		Air	2.62	48.26	2.3	
		Brain	2.62	41.71	2	
	Whole body in steel box	Air	0.67	999.74	0.13	
		Brain	0.67	758.42	5.3	
	Head in air, torso in steel box	Brain (downward detonation)	0.67	820.48	0.15	
		Brain (sideward detontation)	0.67	393.00	0.8	
		Air	1.49	137.90	1.1	
		Brain	1.49	106.87	1.9	
		Air	4.57	17.93	2.2	
		Brain	4.57	11.72	1.9	
	Head in steel box, torso in air	Air	0.67	999.74	0.13	
		Brain	0.67	39.99	1.2	
		Air	1.49	137.90	1.1	
		Brain	1.49	6.21	1.1	
		Air	4.57	17.93	2.2	
		Brain	4.57	0.689	1.2	
Rabbit	Detonation chamber	Air	0.8	382.46	-	(27)
		Brain	0.8	402.07	-	
		Reduction of the number of rapid oscillations between the main pressure peaks Insignificant negative phase between the pressure peaks Slight increase in pressure compared with incident wave				
Rat	Blast tube	Air	-	42	4.5	(31)
		Brain (Ventricle)	-	40	4.5	

lar results were obtained in a study conducted by Yang *et al*, in which sheep were exposed to weak blast waves (37). Apart from lethality, the severity of injury index (SII), calculated by a summation of assigned scores based on the severity of the lesions in various organs, was used to develop damage risk criteria for blast injury. Solid abdominal organs such as the liver appeared to have a higher injury threshold than gas-containing organs in sheep exposed to complex blast waves. It was suggested that injuries in different organs can be induced by different mechanisms (38). Nonetheless, there is a lack of information regarding the injury threshold or criterion for primary blast injury to the brain. A head injury criterion was proposed for blast-induced head acceleration, which was classified as a tertiary blast injury (39). Kato *et al* suggested that the threshold of a shock wave-induced brain injury for the rat is under 1000 kPa (40). While the shock wave in Kato's study was produced by shock wave generator (silver azide micro-explosive ignited by a Nd:YAG laser) and was focused at a specific region in the brain, whether its injury threshold can be compared to that obtained from experiments using a shock tube or detonation is doubt-

ful. The injury threshold for the lung was found to be 2000-10000 kPa (in dogs) in an extracorporeal shock wave lithotripsy study (41) and 2100-2800 kPa (in humans, estimated from sheep and swine data) in studies using shock waves generated by detonation (33). Thus, it is anticipated that the injury threshold of the brain for a primary blast wave would be much lower than 1000 kPa. In any case, an injury criterion useful for blast protection design and safety evaluation remains to be determined.

5 Injury to the peripheral nervous system (PNS)

Studies have been conducted to reveal shock wave-induced injury in the PNS. Blast overpressure can cause axonopathy in central visual pathways and in fibers connecting the retina and midbrain/diencephalon (42). In studies concerning the effect of extracorporeal shock wave therapy (ESWT), the rat paw was subjected to shock wave impulses generated by a lithotripter. Immunohistochemical findings indicated that sensory nerve fibers in the skin were degenerated (43, 44), whereas the expression of the marker for sensory neurons involved with pain perception, calcitonin gene-related peptide (CGRP), was reduced in dorsal root ganglion neurons (45). These results not only accounted for the pain relief by ESWT, but also provided further evidence of damage to the cells of the PNS caused by shock waves. A blast wave can stimulate the vagus nerve, which in turn can elicit dysfunctions of the pulmonary and cardiovascular systems. The vagus nerve is the dominant pathway of the parasympathetic nervous system that controls the function of the lung and heart (46). Previous studies illustrated that bilateral vagal deafferenation completely prevented blast-induced bradycardia, hypotension and apnea in rodents (47-50), providing strong evidence of the involvement of vagal reflexes in primary blast injury. It was noted that pulmonary and cardiac malfunctions developed within 10 seconds after blast. However, arterial baroreflex that is stimulated by a blast wave and triggers an instantaneous response, may not be the mechanism to account for the observed malfunctions in lung and heart (48, 50).

6 Injury to the central nervous system (CNS)

Cellular responses play an important role in brain injury after blast exposure. Morphological examinations provide evidence of degenerative processes in neurons, featured by their darkened atrophic dendrites and accumulation of heavy subunits of neurofilament proteins in the neuronal soma (51-56). Some studies have illustrated that neuronal degeneration and neural disorders after blast were related to the activation of microglia and astrocytes in the brain (51-53, 55). Abundant hypertrophic microglia in the cerebral and cerebellar cortex suggest that blast waves can cause more injury to the surface of the brain than to the deeper regions (52). However, subcortical structures such as the hippocampus were shown to be affected by blast waves as well. Memory and cognitive deficits that were observed in rats after blast exposure (56, 57) resemble the injuries sustained by Soldiers exposed to explosions. Pyknotic nuclei, swollen mitochondria and laminal bodies were present in hippocampal neurons, together with an increase in cytoplasmic vacuoles, 24 hours after blast wave exposure, lasting for 5 days (56, 57). Neurons were shown to undergo apoptotic cell death in response to blast insult. Immediate early genes or oncogenes associated with apoptosis such as c-Jun, c-Myc and c-Fos expressed throughout layers II to VI of the laminar structure of the cerebral cortex, CA1 to CA3 pyramidal cell layer of the hippocampus and granule cell layer of the dentate gyrus were found to occur as early as 2 hours after exposure to short-lasting impulse noise (58, 59). Apoptotic cell death in blast-injured neurons was confirmed by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling staining (TUNEL), which was positive in the cerebral cortex and the hippocampus (58). Interestingly, Kato *et al* (40) found that after exposing the rat brain to shock waves produced by silver azide micro-explosions, the TUNEL-positive cells were exclusively doubled-stained with anti-neuron-specific nuclear protein (NeuN) but not with glial fibrillary acidic protein (GFAP) and 2,3-

cyclic nucleotide 3-phosphohydrolase (CNPase). That is, only neurons underwent apoptosis while astroglial and microglial cells did not. Disruption of axonal transport in neurons after exposure to a blast wave was shown by Saljo *et al* (59, 60). They reported an increase in expression of beta-amyloid precursor protein (β-APP), which is a marker of axonal injury (61), in the thalamus from 6 hours to 21 days post-trauma in the rat brain (59). They also found a marked accumulation of phosphorylated epitope of heavy subunit of neurofilament proteins (p-NFH) in neuronal perikarya from 18 hours to 7 days after exposing rats to shock waves (60). Moochhala *et al* demonstrated a significant performance decrement in coordination and grip strength after exposure to a 21-kPa blast, without injury to the peripheral system (54). This implies that a low-level blast wave can induce mild injury to the CNS. There is confirming evidence of increased nitric oxide generation in the dorsal hippocampus and middle mesodiencephalic reticular formation during the five days post-exposure, as well as the presence of apoptotic neurons in the cerebrum (54). However, neurons around the central canal and the dorsal horn of spinal cord did not appear to be affected (62).

In vitro studies to elucidate injury mechanisms have been carried out using cell cultures. These studies mainly focused on cell injury associated with extracorporeal shock wave lithotripsy. Different cell types, including endothelial cells (63), renal carcinoma cells (64), breast sarcoma cells (65) and leukemia cells (66), were used. The shock wave was found to detach the cells from their substratum, possibly due to shear (64). It also increased membrane permeability or even ruptured the cells. *In vitro* dorsal root ganglion (DRG) cells were more permeable to dye-protein complexes (Evans-blue dye conjugated with albumin) immediately after exposure to blast (67). Changes in cell permeability have been evident in a study using rats, in which transient elevation of NSE (cytosol protein of neuron) and S-100 (cytosol proteins of astrocytes) levels in the cerebrospinal fluid were observed up to 10 hours after exposure to high-energy impulse noise. This implies that the membranes of glial and neuronal cells must have become abnormally permeable (68). This change in permeability can trigger a complex sequence of injuries at the cellular level, resulting in diffuse brain injury (60). In agreement with *in vivo* findings, reduction in cell viability, pyknotic or karyorrhectic nuclei, vacuolization and destruction of cytoskeletal structures were observed in cells exposed to shock waves (63-66). It is noteworthy that cytoskeletal damage in renal cells caused by shock wave recovered within 24 hours after shock wave exposure (64). The cytoskeleton is important for maintaining the shape of cell. It has been suggested that a shock wave can rupture and depolymerize the cytoskeletal fibers, leading to blebs formation as well as increased cell permeability (64). A study conducted by Suneson *et al* demonstrated blast-induced injuries in DRG cells in culture (67). The cells were grown on a culture plate which was kept in a rubber tube filled with water and a pressure wave (with a peak value of 206.8 kPa) was induced by the impact of a high-energy missile against the tube. Light microscopy and electron microscopy revealed neuronal damage as described previously at 6 hours post-trauma. Moreover, discontinuities of neurofilaments in nerve processes, extensive changes in microtubules, and neurofilament tangles in neurons were observed. Many *in vitro* systems have been developed to study brain injury caused by mechanical insults. Brain cells or organotypic slices have been cultured on Petri dishes or stretchable membranes, which were then subjected to weight drop, hydrostatic pressure, acceleration, stretching or transection (69). Among all the mechanical means of producing injuries, fluid percussion barotraumas resemble overpressure-induced injury most accurately, despite its longer duration compared with that of the shock wave. Shepard *et al* built a fluid percussion device, which delivered a pressure pulse ranging from 48.3 to 406.8 kPa with a duration of 20 to 30 ms (70). Cellular injuries, such as reduction of cellular viability and increased production of leukotriene C_4 were observed in human glial cells after fluid percussion injury. This study, together with that of Suneson *et al*, strengthened the hypothesis that blast over-

pressure is a cause of brain injury.

Although the mechanism of shock wave-induced brain trauma is still unclear, a few researchers have proposed diagnostic and preventive measures against the injury. Blast-induced injury in the CNS leads to changes in biochemical parameters in blood. These biomarkers included ions, antioxidants, hormones, eicosanoids and anticoagulants (14, 71, 72), which could be used as clinical indicators of the severity of brain damage after blast. In a study exposing rat brains to a shock wave generator, Nakagawa *et al* showed that a Gore-Tex dural substitute over the dura mater attenuated the shock wave propagation by 96% and eliminated tissue damage (73). Pharmacologically, morphine was proven to be effective in maintaining arterial blood pressure and heart rate by attenuating the blast-induced vagal reflex (50). Another neuroprotective agent, aminoguanidine, facilitated the recovery of coordination and grip strength in rats after blast exposure. It also reduced the number of degenerating cortical neurons through inhibiting nitric oxide generation (54).

7 On-going studies on cellular injury caused by overpressure/shock wave

We have established an *in vitro* model for studying overpressure-induced brain injury. A barochamber was designed and fabricated to generate pressure waves of high amplitude and short duration (see Figure 2a). Preliminary studies were conducted to elucidate the effects of overpressure on astroglial cells. Expressions of genes for apoptosis (bax, caspase 3 and caspase 8 and Fas-ligand), reactivity (mitogen activated protein kinase kinase 1, Nestin, GFAP and vimentin) and survival (bcl-2, GDNF, IL-3) were examined at 24, 48 and 72 hours post-trauma. An overpressure with a magnitude of 158.7 ± 34.5 kPa and duration of 10.98 ± 1.40 ms caused elevated levels of reactivity and survival gene expression at 24 hours post-trauma. By 48 hours, a decreased expression of apoptotic genes was demonstrated. Live/Dead viability/ cytotoxicity staining and Vybrant apoptosis staining showed that the astrocytes survived the insult without undergoing apoptosis (74). In addition to the *in vitro* study, we have recently carried out an animal study in which rats were exposed to shock waves generated by a shock tube. The peak overpressure was 158.6 kPa and the duration was 10 ms. Twenty-four hours after exposure to the shock wave, the brain was harvested and immunostained for GFAP. Consistent with our *in vitro* study, the immunoreactivity of GFAP was significantly pronounced in the rat brain, particularly in the hippocampus, after exposure as shown in Figure 3 which contains new data.

Astrocytes are essential for neuronal metabolic, antioxidant, and trophic support, as well as for normal synaptic function. The interaction between neurons and astrocytes appears to play a crucial role in traumatic brain injury, in which reactive astrocytes contribute to the preservation of neural tissue and restriction of inflammation (75). Saljo *et al* illustrated the dose-dependent increase of astroglial activation in certain regions of the rat brain after exposure to high impulse noise (55). *In vitro* models of overpressure-induced brain injury would allow precise control over the testing conditions and the mechanical stimulus. It also facilitates the examination of post-traumatic responses of different brain cell types. Further *in vitro* studies will focus on the interplay between different populations of brain cells especially neurons, microglia and astrocytes, when exposed to transient overpressure This research will provide new insights into the injury pathways triggered by a blast wave.

8 Discussion

Studies associated with blast wave-induced neurotrauma are scarce in the literature and they have primarily been concerned with the systemic effects of the blast, rather than the mechanism of injury. The mechanism of blast-related CNS injury was suggested to be similar to that of other head injuries (56). Non-blast related traumatic brain injury (TBI) is known to initiate a complex sequence of destructive and neuroprotective cellular responses and the initial mechanical injury is followed by an extended time period of secondary brain damage (76). It is thought that the

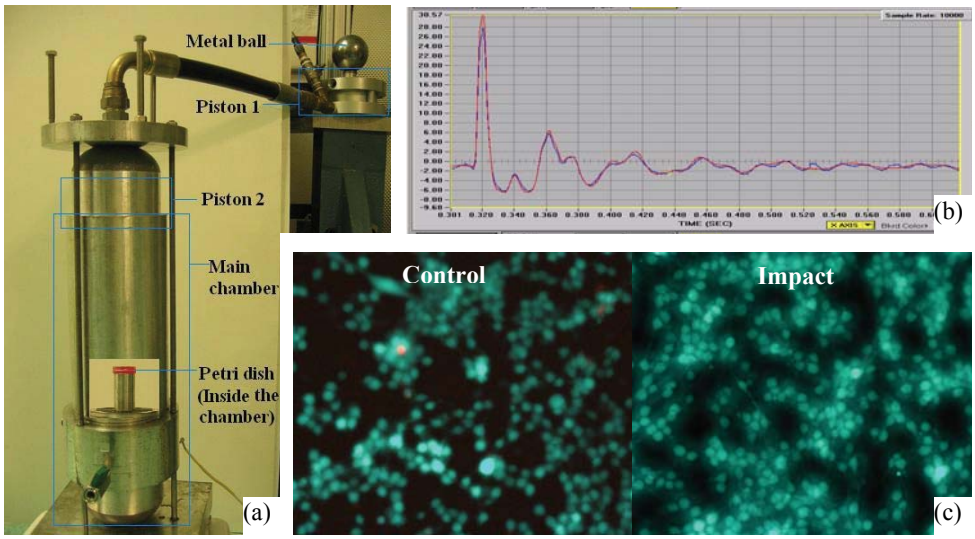

Figure 2: (a) Barochamber, consisting of a driving cylinder (Piston 1), a hose, an ultra-low mass piston (Piston 2) and the main chamber. The Petri dish resides on the pedestal inside the chamber. The entire system is filled with distilled warm (37°C) water. (b) A pressure wave, of high magnitude and short duration is produced when the metal ball strikes the rod of the driving cylinder. (c) Live/Dead viability/ cytotoxicity staining (red indicates dead cells and green indicates living cells) of C6 cells 24 hours after overpressure insult (Left: Control group; Right: Overpressure group)

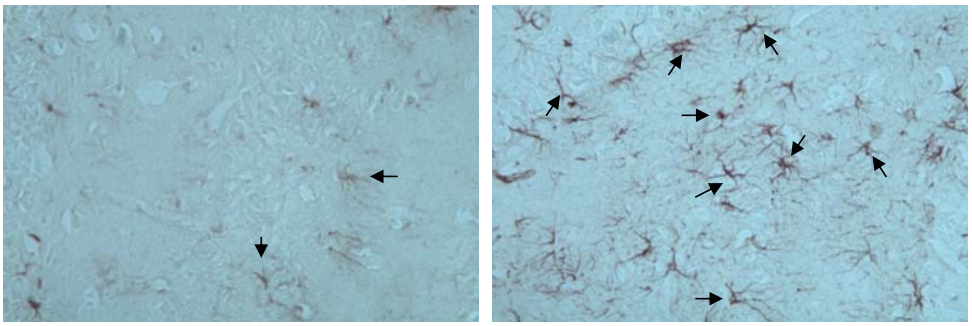

Figure 3: In the hippocampus, GFAP immunoreactivity is higher in the overpressure group (Right) than in the Control group (Left). Arrows indicate GFAP positive cells; Magnification: 400X

primary phase of tissue disruption instigates secondary injury processes causing pathophysiological changes in the brain. As a consequence of the initial mechanical impact to the head, cerebral metabolism, blood flow and ion homeostasis are altered for a period of hours to months (77). During the secondary injury, high levels of glutamate, calcium and lactate are released (78). Cytokines are generated, leading to an inflammatory response that aggravates tissue damage (79). Jarell *et al* indicated that most brain dysfunctions are the result of secondary neuronal damage and not the primary mechanical insult (80). Besides the destructive processes, neuroprotective events in repair and regeneration also take place (81). However, harmful processes usually overshadow and eventually lead to tissue loss due to cell death. Another downstream mechanism which has been established to play a role in TBI is oxidative stress or the overproduction of reactive oxygen species (82). The main difference between traumatic brain injuries due to impact and those due to blast

is that direct impact on the head results in pressure gradients within the brain and rotational effects causing strain (83), whereas blast waves may only involve propagation of stress waves inside the skull without any large head motions. However, data in the literature demonstrating the biomechanics of brain injury during exposure to blast wave are very limited. Another suggested mechanism is vascular disturbances in the brain tissue associated with compression and decompression of the atmosphere during explosion, leading to arterio-capillary anemia, venous congestion and rupture of delicate walled vessels. This hypothesis was rejected by Hooker (84), who conducted a detailed animal study regarding air concussion.

In spite of decades of study on blast related neurotrauma, there are some issues and limitations which impede the progress of researches in this field. First of all, there are no well-established protocols for conducting the study. The magnitude of overpressure ranged from 2.8 to as high as 4998.7 kPa, with the durations ranging from 0.35 to 52 ms in the aforementioned studies. The parameters quantifying the blast or shock waves were usually not well-explained. Their effects on brain injury need to be elucidated more systematically. The studies by Richmond *et al* are good examples to follow, in which they examined the relationship between lethality and the parameters of the blast wave (33), as well as the biological responses to complex waves (simulating an enclosed space such as a vehicle) (85) and repeated waves (36). Secondly, there is a lack of understandings of shock wave physics among medical researchers. While most of the studies were published in medical or physiological journals, the authors seldom justified their experimental designs. The pressure terminology is confusing since many authors did not specify whether the reported pressure was the incident pressure, reflected pressure or dynamic pressure. According to Bowen *et al* (32), the orientation of the person relative to the blast wave front determines which pressure among the three is the most effective pressure affecting biological responses. For instance, a person is only loaded with incident pressure if the long axis of the body is parallel to the blast wave front. If the long axis of the body is perpendicular to the wave front, the person is exposed to both the incident pressure and the dynamic pressure. If he/she is near a reflected surface, the reflected pressure would be the effective pressure (32, 33). Furthermore, details of the experimental setup such as the location of the experimental animal inside the shock tube and the type of fixture used to hold the animal should be designed carefully so that the animal is subjected to the desired waveform of the shock wave and reflection of the wave from the fixture is minimized to avoid complex blast waves. These considerations require a basic understanding of shock wave physics.

It is vital for future research in this area to focus not only on the cellular aspects, but also the overall biomechanics of the brain due to shock wave exposure. Advancements in pressure transducer design in terms of frequency response and miniaturization, enables the researcher to obtain pressure data inside the brain of a small animal (31) with minimal damage to the brain tissue. Thus, the transmission pattern of pressure waves inside the brain can be recorded. Well established finite element models of the head (86, 87), together with the use of computational fluid dynamics modeling techniques would be useful for estimating the local strain or intracranial pressure distribution inside the brain when the head is exposed to a blast wave. The key biomechanical question is the mechanism of injury in the absence of high linear and angular accelerations of the head. That is the pressure mechanism proposed over a half a century ago by Gurdjian *et al* (88) needs to be clearly identified and documented.

9 Conclusions

The increasing incidence of mild TBI in returning veterans has aroused the attention of researchers toward blast-induced brain injury. Decades of study have been conducted to investigate the effects of primary blast wave on the CNS. Transmission of pressure wave into the brain during blast was demonstrated. Neuronal injury, microglial and astroglial cell activation were evident after exposure to overpressure. Nevertheless, the injury

mechanisms at both the macroscopic and molecular level are still undefined and injury criteria still remain to be determined. The slow progress may be due to the lack of well-established protocols and knowledge of shock wave physics among medical researchers. Research on cellular injury of the brain induced by blast wave is essential for the development of more effective treatment modalities and for the identification of diagnostic biomarkers. Together with biomechanical studies on the brain during blast exposure, these findings can make a significant contribution towards the determination of new injury criteria for blast-related brain injury. After the injury mechanisms and tolerances have been established, effective protective countermeasures can be found.

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